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(54) Method for purification and transfer to separation/detection systems of DNA sequencing samples and plates used therefor

(57) Disclosed are: a part for assembling reaction vessels comprising a base plate with through-holes passing said plate in the direction of thickness; a method for preparation of reaction vessels containing a reaction solution characterized in that the part is immersed in a reaction solution to charge the through-holes with the reaction solution and then one of apertures of each hole of the above through-holes is sealed with a membrane to complete said reaction vessels; and a plate comprises a base plate with through-holes passing said plate in the direction of thickness and a membrane for sealing one of apertures of each hole of said through-holes. The plate is used for purification of DNA sequencing samples using a pressure difference or an electrical potential, and transferring DNA sequencing samples to a separation/detection system. By the means or the method of the invention, reaction solutions can be easily charged into a large number of wells in a short time, unreacted labeling substances and the like can be easily removed in a short time from a plurality of DNA sequencing samples, and a large number of DNA sequencing samples can be easily purified and/or transferred to electrophoresis capillaries in a short time.

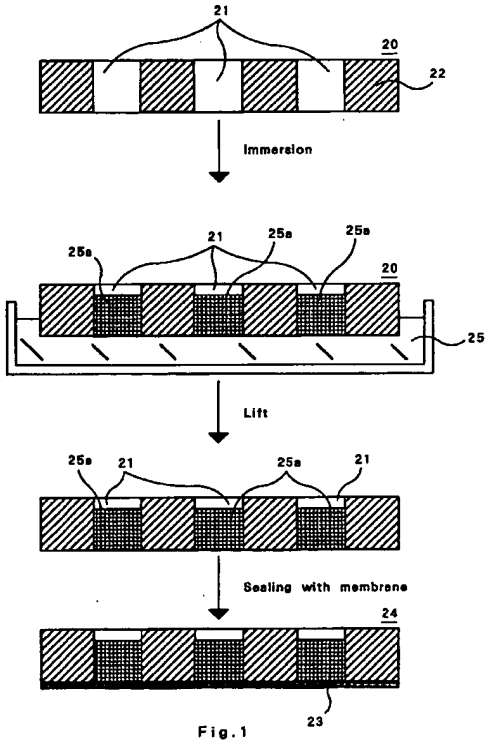

Fig.1

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Description

BACKGROUND OF THE INVENTION

The present invention relates to a plate used for transferring DNA sequencing samples to a separation/detection system. The plate of the present invention is capable of transferring at one time (at the same time) a plurality of reaction products produced in wells of a multi-reaction plate to an analyzer using a large number of capillaries, such as an analyzer based on electrophoresis.

The present invention further relates to a method for transferring DNA sequencing samples utilizing the plate mentioned above, a method for purifying DNA sequencing samples utilizing the same and a method for purifying and transferring DNA sequencing samples utilizing the same.

With recent marked developments of DNA detection methods utilizing laser fluorescence, laser fluorescence DNA sequencers have been widely used as very useful apparatuses. Developments of such techniques have also enabled analysis of a large number of samples in, for example, genome researches and DNA diagnoses (approximately 100 lanes or less/2 operations/day/apparatus). Typical examples of such laser sequencers are those utilizing slab type gels and capillary type gels.

Further, in order to analyze samples of a further larger number than is possible by current techniques, apparatuses having a larger number of electrophoresis lanes (for example, 200 to 1000 lanes/operation) are under developing. As examples of such apparatuses, multi-capillary type sequencers can be mentioned.

However, as the number of lanes increases, the operation of transferring samples to the lanes becomes more time-consuming and more burdensome. That is, when a large number of samples are loaded on each of capillary gels, each sample should be injected into each capillary by contacting one end of the capillaries with a fine electrode lead. Therefore, development of a technique enabling short-time and easy transfer of a large number of samples is considered an urgent requisite.

Further, charging reaction solutions into wells of micro-titer plates also requires more time as the number of the wells increases. Therefore, a means for charging reaction solutions into wells in a short time is also desired.

Furthermore, in DNA sequencing reaction products, fragments of various lengths labeled with fluorescent substance or the like are present with unreacted labeling reagent. A major part of such coexisting unreacted labeling reagent has not been utilized in the reaction and exists in the reaction mixture in free form. If such a reaction solution is used for electrophoresis as it is, the fluorescence label of a high concentration is simultaneously migrated and generates a signal markedly stronger than those of the target sequences. As a result, intended analysis becomes impossible to be performed. Accordingly, the fluorescence label should be removed before the separation. However, removal of the unreacted labeling reagent with respect to hundreds of samples requires much labor and time. As a result, even if efficiency of DNA sequencing methods itself is improved, a rate-determining factor would be present before the sequencing.

Therefore, the first object of the present invention is to provide a means capable of easily charging reaction mixtures into a large number of wells in a short time.

The second object of the invention is to provide a method for easily removing unreacted labeling substances and the like in a short time from a plurality of DNA sequencing samples containing the labeling substances.

The third object of the present invention is to provide a means capable of easily transferring a large number of DNA sequencing samples to electrophoretic capillaries in a short time.

The fourth object of the present invention is to provide a method for easily removing unreacted labeling substances and the like in a short time from a plurality of DNA sequencing samples containing the labeling substances and capable of easily transferring a large number of DNA sequencing samples to electrophoretic capillaries in a short time.

SUMMARY OF THE INVENTION

The first embodiment of the present invention relates to a part for assembling reaction vessels comprising a base plate with through-holes passing said plate in the direction of thickness.

The second embodiment of the present invention relates to a method for preparation of reaction vessels containing a reaction solution characterized in that the above part of the present invention is immersed in a reaction solution to charge the through-holes with the reaction solution and then one of apertures of each hole of the above through-holes is sealed with a membrane to complete said reaction vessels.

The third embodiment of the present invention relates to a plate used for transferring DNA sequencing samples to a separation/detection system characterized in that the plate comprises a base plate with through-holes passing said plate in the direction of thickness and a membrane for sealing one of apertures of each hole of said through-holes.

The fourth embodiment of the present invention relates to a method for removal of unreacted low molecular compounds contained in DNA sequencing samples characterized in that said DNA sequencing samples are charged in the through-holes of the plate according to the third embodiment of the present invention respectively, a pressure difference is provided between the remaining apertures of the through-holes and the outside of the sealing membrane in such a manner that the membrane side should have negative pressure to transfer said unreacted low molecular compounds in the

DNA sequencing samples to the outside of the samples through said membrane.

The fifth embodiment of the present invention relates to a method for removal of unreacted low molecular compounds contained in DNA sequencing samples characterized in that said DNA sequencing samples are charged in the through-holes of the plate according to the third embodiment of the present invention respectively, and a potential is applied between said DNA sequencing samples and the outside of the sealing membrane to transfer said unreacted low molecular compounds in the DNA sequencing samples to the outside of the samples through said membrane.

The sixth embodiment of the present invention relates to a method for transferring DNA sequencing samples respectively charged in the through-holes of the plate according to the third embodiment of the present invention to electrophoretic capillaries for a separation/detection system wherein one of terminals of each capillary of the electrophoretic capillaries are inserted into said DNA sequencing samples and a potential is applied between said electrophoretic capillaries and the outside of the sealing membrane to transfer said DNA sequencing samples into the electrophoretic capillaries.

The seventh embodiment of the present invention relates to a method for transferring DNA sequencing samples to a separation/detection system characterized in that said DNA sequencing samples are charged in the through-holes of the plate according to the third embodiment of the present invention respectively, one of terminals of each capillary of the electrophoretic capillaries are inserted into said DNA sequencing samples contained in the through-holes from the unsealed apertures and the charged contents are aspirated into the electrophoretic capillaries from the other terminal of each capillary of the electrophoretic capillaries to transfer target substances contained in said DNA sequencing samples into the electrophoretic capillaries.

The eighth embodiment of the present invention relates to a method for purification and transfer to a separation/detection system of DNA sequencing samples, which comprises removing unreacted low molecular compounds contained in the DNA sequencing samples and then transferring target substances contained in the DNA sequencing samples to electrophoretic capillaries for the separation/detection system, characterized in that said DNA sequencing samples are charged in the through-holes of the plate according to the third embodiment of the present invention respectively, one of terminals of each capillary of electrophoretic capillaries are inserted into said DNA sequencing samples, a potential is applied between the outside of said membrane sealing the through-holes charged with said DNA sequencing samples and the other terminal of each capillary of the electrophoretic capillaries to transfer unreacted low molecular compounds contained in said DNA sequencing samples to the outside of the samples through the membrane, and then a potential is applied between the outside of said membrane and the other terminal of each capillary of the electrophoretic capillaries to transfer said target substances in the DNA sequencing samples into the electrophoretic capillaries.

The ninth embodiment of the present invention relates to a method for purification and transfer to a separation/detection system of DNA sequencing samples, which comprises removing unreacted low molecular compounds contained in the DNA sequencing samples and then transferring target substances contained in the DNA sequencing samples to electrophoretic capillaries for the separation/detection system, characterized in that said DNA sequencing samples are charged in the through-holes of the plate according to the third embodiment of the present invention respectively, a pressure difference is provided between the remaining apertures of the through-holes and the outside of the sealing membrane in such a manner that the membrane side should have negative pressure to transfer said unreacted low molecular compounds in the DNA sequencing samples to the outside of the samples through said membrane, then one of terminals of each capillary of electrophoretic capillaries are inserted into said DNA sequencing samples, and a potential is applied between the outside of said membrane sealing the through-holes charged with said DNA sequencing samples and the other terminal of each capillary of the electrophoretic capillaries to transfer said target substances contained in said DNA sequencing samples into the electrophoretic capillaries.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a schematic cross-sectional view for illustrating a part for assembling reaction vessels of the present invention and the method for preparation of the vessels of the present invention containing a reaction mixture.

Figure 2 is a schematic cross-sectional view of a plate for transferring DNA sequencing samples to a separation/detection system of the present invention.

Figure 3 is a schematic cross-sectional view for illustrating the method for removal of unreacted low molecular compounds according to the present invention (pressure difference method).

Figure 4 is a schematic cross-sectional view for illustrating the method for removal of unreacted low molecular compounds (electrical method) and the method for transferring DNA sequencing samples to a separation/detection system (electrical method) according to the present invention.

Figure 5 is a schematic cross-sectional view for illustrating the method for removal of unreacted low molecular compounds according to the present invention (electrical method).

Figure 6 is a schematic cross-sectional view for illustrating the method for transferring DNA sequencing samples to a separation/detection system according to the present invention (pressure difference method).

Figure 7 is a schematic view for illustrating the method for separation and detection by electrophoresis.

Figure 8 is a schematic view for illustrating the method for removal of unreacted low molecular compounds (pressure differentiation method).

Figure 9 is a schematic view for illustrating the method for transferring DNA sequencing samples to electrophoretic capillaries (electrical method).

Figure 10 shows a sequencing ladder of a migration pattern obtained by an imaging analyzer.

PREFERRED EMBODIMENTS OF THE INVENTION

Part for assembling reaction vessels and method for preparing the same (the first and the second embodiments).

The part for assembling reaction vessels of the present invention consists of a base plate with through-holes passing the plate in the direction of thickness. The part can be used for the preparation of reaction vessels containing a reaction solution. It will be explained hereinafter by the reference to Fig. 1.

The part for assembling reaction vessels 20 consist of a base plate 22 with through-holes 21 passing the plate in the direction of thickness. The base plate 22 can be made of, for example, synthetic resins, glass or the like. The size and the thickness of the plate can be suitably selected considering the size (inner-diameter and depth) and the number of the through-holes 21. The size (inner-diameter and depth) and the number of the through-holes 21 can be defined properly depending on the use of the plate. The inner-diameter of the through-holes 21 may be, for example, 0.05 to 10 mm, preferably 0.5 to 5 mm. The depth of the through-holes 21 may be, for example, 0.2 to 200 mm. The number of the through-holes 21, although the larger it is, the more samples can be processed simultaneously, is properly selected considering the number of electrophoretic capillaries, the performance and the like of a separation/detection apparatus to be used.

Vessels containing a reaction solution can be prepared by immersing the above-mentioned part for assembling reaction vessels 20 in a reaction solution 25 to charge the through-holes 21 with the solution 25a, and then sealing one of the apertures of each through-hole with a membrane 23 to obtain the reaction vessel 24.

Nature of the reaction solution is not particularly limited and it can be selected depending on the purpose. For example, it can be a buffer solution containing various kinds of enzymes.

Plate for transfer to a separation/detection system (the third embodiment)

The plate for transfer to a separation/detection system of the present invention will be explained by the reference to Fig. 2.

A plate for transfer to a separation/detection system 1 consists of a base plate 3 with through-holes 2 passing the plate in the direction of thickness and a membrane 5 provided for sealing one of apertures 4 of each through-hole 2.

The plate 3 can be made of, for example, synthetic resins, glass or the like and the size and the thickness thereof can be suitably selected considering the size (inner-diameter and depth) and the number of the through-holes 2.

The size (inner-diameter and depth) and the number of the through-holes 2 can also be suitably selected considering the use of the plate. The inner-diameter of the through-holes 2 may be, for example, 0.1 to 5 mm, and the depth of the through-hole may be for example 0.2 to 200 mm. The number of the through-holes 2, although the larger it is, the more samples can be processed simultaneously, is properly selected depending on the number of electrophoretic capillaries, the performance and the like of a separation/detection system to be used.

Material of the membrane 5 can be suitably selected depending on the use of the plate. For example, when target substances in DNA sequencing samples are electrically transferred to the electrophoretic capillaries, the membrane 5 can be made of a material capable of conducting electricity when contacting with an electrolyte. For example, it can be a membrane used as a separator membrane for molecular sieving such as ultrafiltration membranes.

When target substances in DNA sequencing samples are transferred to the electrophoretic capillaries by utilizing a pressure difference, the membrane 5 may be a liquid permeating material, such as a membrane of cellophane, polyether sulfone or the like.

When unreacted low molecular compounds in the DNA sequencing sample are electrically removed from the system through the membrane 5, the membrane 5 can be a material which permeates the low molecular compounds but not reaction products, i.e., DNA fragments. For example, an ultrafiltration membrane can be used.

Further, when the unreacted low molecular compounds in the DNA sequencing samples are removed electrically from the system through the membrane 5 and then the target substances in the DNA sequencing samples are electrically transferred to the electrophoretic capillaries, the membrane 5 can be a material which permeates the low molecular compounds but not the reaction products, i.e., DNA fragments, and which is capable of conducting electricity when contacting with an electrolyte. As such a membrane, for example, an ultrafiltration membrane can be employed.

As the ultrafiltration membrane of the plate of the present invention, a polyether sulfone membrane can be used.

Method for removing unreacted low molecular compounds (Pressure difference method) (the forth embodiment)

The method will be explained hereinafter by the reference to Fig. 3.

As the through-holes 2 of the plate 1 of the present invention are charged with DNA sequencing samples 7, a pressure difference is provided between the apertures 2a of the through-holes and the outside of the membrane 5 so that the outside of the membrane 5 should have negative pressure. More specifically, the pressure difference can be produced so that the outside of the membrane 5 should have negative pressure by providing a vacuum container 20 on the plate 1 at the membrane side and reducing the pressure therein. The unreacted low molecular compounds in the DNA sequencing samples can be transferred to the outside of the samples through the membrane 5 together with the other low molecular compounds such as water.

Method for removing unreacted low molecular compounds (electrical method) (the fifth embodiment)

The fifth embodiment may be exemplified by the following two methods.

In the first method, terminals of electrophoretic capillaries are inserted into the DNA sequencing samples respectively, wherein the other terminals of said capillaries are contacted with an electrolyte having an electrode (1); the sealing membrane are contacted with an electrolyte with which an electrode (2) is contacted; and a potential is applied between the electrodes (1) and (2). This method in which an electrode is immersed in the DNA sequencing sample is shown in Fig 5.

As an electrode 12 is immersed into the DNA sequencing sample 7 charged in the through-hole 2 of the plate 1, a potential is applied between the outside of the membrane 5 and an electrode 12 so that the electrode 12 should be a cathode. In this manner, the unreacted low molecular compounds in the DNA sequencing sample 7 can be transferred to the outside of the sample 7 through the membrane 5 as well. Although Fig. 5 shows the above-described operation for only one of the through-holes 2, the operation of transferring the low molecular compounds can be carried out for a plurality of or all of the through-holes 2 simultaneously.

In the above-mentioned method, unreacted low molecular compounds in the DNA sequencing samples within a plurality of or all of the through-holes are simultaneously transferred through the membrane 5 to the outside of the samples by contacting the whole surface of the membrane 5 of the plate 1 with the electrolyte 8, immersing each electrode 12 or each capillary 6 of electrophoretic capillaries in the sample within each of the through-holes and applying a potential.

In the second method, electrodes are inserted into the DNA sequencing samples respectively; the sealing membrane are contacted with an electrolyte with which an electrode is contacted; and a potential is applied between the electrodes inserted in the DNA sequencing samples and the electrode contacted with the electrolyte. This method will be explained by the reference to Fig. 4.

As one of the terminals 6a of each electrophoretic capillary 6 is immersed into the DNA sequencing sample 7 charged in each through-hole 2 of the plate 1, a potential is applied between the outside of the membrane 5 and the other terminal 6b of the capillary 6 so that the other terminal 6b of the capillary 6 should be a cathode. The potential can be applied by using an electrode 9 as an anode and an electrode 10 as a cathode and contacting the membrane 5 with an electrolyte 8 where the electrode 9 is immersed and the other terminal 6b of the electrophoretic capillary 6 with an electrolyte 11 where the electrode 10 is immersed.

In this manner, the unreacted low molecular compounds within the DNA sequencing sample 7 can be transferred to the outside of the sample through the membrane 5. Although Fig.4 shows the above-described operation for only one of the through-holes 2, the operation of transferring the low molecular compounds can be carried out for a plurality of or all of the through-holes 2 simultaneously.

Method for transfer to separation/detection system (electrical method) (the sixth embodiment)

This method is for transferring the DNA sequencing samples charged in the through-holes of the plate of the present invention to each of the electrophoretic capillaries for separation and detection. It will be explained by the reference to Fig. 4.

As one of terminals 6a of each capillary 6 of electrophoretic capillaries is immersed into the DNA sequencing sample 7 charged in the through-hole 2, a potential is applied between the outside of the membrane 5 and the other terminal 6b of the capillary 6. The potential can be applied, as shown in Fig. 4, between the electrode 9 used as a cathode and the electrode 10 used as an anode, while the membrane 5 is contacted with an electrolyte 8 wherein the electrode 9 is immersed and the other terminal 6b of the capillary 6 is contacted with an electrolyte 11 wherein the electrode 10 is immersed.

This electrical injection method can be used in either case, whether the charged content of the electrophoretic capillaries (separation system) consists of a polymer solution or an acrylamide gel. An electric voltage of 1 to 10 kV is applied between the electrode 9 and the electrode 10 for one to several tens seconds to carry out the injection electrophoretically.

Thus, the target substances in the DNA sequencing sample 7 can be transferred to each of the electrophoretic capillaries 6 (around the terminal 6a).

Although the Fig. 4 shows the above-mentioned operation for only one of the through-holes 2, the target substances in the DNA sequencing samples within a plurality of or all of the through-holes are transferred into

the capillaries simultaneously, by contacting the whole surface of the membrane 5 of the plate 1 with an electrolyte 8 and placing the other terminal 6b of each electrophoretic capillary 6 on the anode side.

Method for transfer to separation/detection system (Pressure difference method) (the seventh embodiment)

This method is for transferring the DNA sequencing samples charged within the through-holes of the plate of the present invention to each of the electrophoretic capillaries for separation and detection, and it will be explained by the reference to Fig. 6.

As the DNA sequencing sample 7 is charged in the through-holes 2 of the plate 1, one of terminals 6a of each capillary of electrophoretic capillaries 6 is immersed in the DNA sequencing sample 7 in the through-hole 2 from the aperture of the through-hole 2. Then, the charged content 6c (for example, a polymer solution) in the electrophoretic capillary 6 is aspirated from the other terminal 6b of the electrophoretic capillary 6. Thus, the target substances contained in the DNA sequencing sample 7 can be transferred into the electrophoretic capillary 6.

The target substances contained in the DNA sequencing samples 7 of every through-holes 2 can be transferred to each of electrophoretic capillaries simultaneously by immersing each of electrophoretic capillaries 6 into each of the through-holes 2 of the plate 1 respectively and aspirating them.

Method for transfer to purification/separation/detection system (the eighth embodiment)

This method is for removing unreacted low molecular compounds in the DNA sequencing samples and then transferring the target substances in the DNA sequencing samples to electrophoretic capillaries for separation and detection. In this method, the above-mentioned method for removing unreacted low molecular compounds contained in the DNA sequencing samples and the above method for transferring the DNA sequencing samples to separation/detection system are carried out successively.

The method of the fifth embodiment of the present invention is used for removing low molecular compounds.

Namely, as shown in Fig.4, as one of the terminals 6a of each capillary 6 of electrophoretic capillaries is immersed into the DNA sequencing sample 7 charged in the through-hole 2 of the plate 1 of the present invention, a potential is applied between the outside of the membrane 5 and the other terminal 6b of the capillary 6 so that the other terminal 6b should be a cathode to transfer the unreacted low molecular compounds in the DNA sequencing sample 7 to the outside of the sample through the membrane 5.

Then, the method of the sixth embodiment of the present invention is employed for transfer of the sample.

That is, the target substances in the DNA sequencing sample 7 are transferred to the electrophoretic capillary 6 by applying a potential between the outside of the membrane 5 and the other terminal 6b of each capillary 6 of the electrophoretic capillaries so that the other terminal 6b should be an anode.

Removal of the low molecular compounds and transfer of the target substances can readily be conducted by reversing the polarity of the electrodes 9 and 10. However, the electrolyte 8 containing the unreacted low molecular compounds transferred through the membrane 5 is preferably changed before the transfer of the target substances in order to prevent the low molecular compounds from returning into the sample 7.

Method for transfer to purification/separation/detection system (the ninth embodiment)

This method is for removing the unreacted low molecular compounds in the DNA sequencing samples and transferring the target substances in the DNA sequencing samples to electrophoretic capillaries for separation and detection. In this method, the above-mentioned method for removing unreacted low molecular compounds in the DNA sequencing samples and the above method for transferring the DNA sequencing samples to a separation/detection system are carried out successively.

The method of the fourth embodiment of the present invention is employed for removing low molecular compounds.

Namely, as shown in Fig.3, as the through-holes 2 of the plate 1 of the present invention are charged with DNA sequencing sample 7, a pressure difference is provided between the apertures 2a of the through-holes and the outside of the membrane 5 so that the outside of the membrane 5 should have negative pressure. More specifically, the pressure difference can be provided so that the outside of the membrane 5 should have negative pressure by providing a vacuum container 20 on the plate 1 at the membrane 5 side and reducing the pressure therein. The unreacted low molecular compounds in the DNA sequencing sample can be transferred to the outside of the sample through the membrane 5 together with the other low molecular compounds such as water.

Then, the method of the sixth embodiment of the present invention is used for transfer of the sample.

That is, as shown in Fig.4, as one of the terminals 6a of each capillary 6 of electrophoretic capillaries is immersed into the DNA sequencing sample 7 charged in the through-hole 2 of the plate 1 of the present invention, a potential is applied between the outside of the membrane 5 and the other terminal 6b of the capillary 6 to transfer the target substances in the DNA sequencing sample 7 to the inside of the electrophoretic capillaries 6.

The DNA sequencing samples, the object to be

dealt in the present invention, may be, for example, products of a large number of chemical reactions or enzymatic reactions (96 or more reactions/plate/operation) produced in a large number of spaces (wells, holes etc.). Nature, number and the like of the reactions are not particularly limited. For example, the DNA sequencing samples may be products of DNA sequencing reactions carried out in the through-holes of the plate of the present invention or products of the DNA sequencing reactions carried out in wells of a conventional microtiter plate. Further, as the DNA sequencing reactions, Sanger reactions using dideoxynucleotides, DNA cycle sequencing conducted by using PCR (polymerase chain reaction) and the like can be exemplified.

When the DNA sequencing reactions are carried out in the spaces formed in the through-holes of the plate of the present invention, the following procedure is preferably employed. First, reagents and enzymes, other than those required to be of different kind for each through-hole (for example, template DNA and the like), such as reaction enzyme (thermostable polymerase or the like), reaction buffer and the like are introduced together into each through-hole of the part for assembling reaction vessels of the first embodiment of the present invention as explained in the second embodiment of the present invention. That is, a certain predetermined amount of a reagent and/or enzyme solution can be introduced into each of the through-holes simply by immersing the part for assembling reaction vessels according to the first embodiment of the present invention into the reagent and/or enzyme solution and pulling the part up from the solution. When the plate is provided with two or more kinds of holes with different sizes, reaction vessels capable of inclusion of different amounts of reagent and/or enzyme solution can be formed. Then, a membrane is applied to one surface of the plate of the part to complete a plate retaining the reagent and/or enzyme solution in its through-holes.

The obtained plate can be used for desired reactions such as DNA sequencing reactions or the like.

As the reactions carried out in the vessels, sequencing reactions performed at 37°C and utilizing DNA polymerase are advantageous because of the reaction solution retaining property of the membrane. This technique of the present invention extremely simplify the procedure of first charging of the reagent and/or enzyme solution into multiple reaction spaces.

However, instead of using this technique, the reagent and/or enzyme solution may be charged one by one.

The target substances transferred to electrophoretic capillaries by the method of the present invention can be separated and detected by conventional methods. As such conventional methods, the DNA sequencing technique based on the DNA capillary electrophoresis can be mentioned. As a DNA separation system used for the DNA capillary electrophoresis, for example, the capillary lanes 31 provided in a grid-like arrangement where the capillary lanes are provided three-dimensionally regularly (for example, like parallel columns) as shown in Fig. 7 can be used. The grid-like arrangement of the capillaries can be correspond to the arrangement of the through-holes 33 of the multi-through-hole plate 32 of the present invention so that each capillary should be rightly inserted into each corresponding through-hole 33 of the multi-through-hole plate 32.

The membrane (not shown) provided on the bottom surface of the multi-through-hole plate 32 provided with the capillaries is contacted with the upper surface of buffer contained in a cathode electrophoretic bath 34. A potential is applied between the cathode electrophoretic bath 34 and an anode electrophoretic bath 35 in which the other terminal of each capillary is immersed by means of an electric source 36. Thus, all of the target substances in the multi-through-hole plate 32 are electrophoretically injected into the capillaries. Then, the target substances are separated electrophoretically and separated fragments are detected by a detector 37. The detector 37 is selected depending on the nature of the marker charged on the fragments. For example, when the marker is a fluorescence marker, a fluorescence detector is used, and an imaging analyzer can be used for an RI marker.

EXAMPLES

The present invention will be further illustrated by the reference to the following examples.

In the following examples, unreacted low molecular compounds contained in DNA sequencing samples were removed by using an apparatus as shown in Figure 8, and then DNA sequencing samples were transferred to electrophoretic capillaries by using a capillary electrophoresis apparatus as shown in Figure 9. Further, separation and detection were performed by a separation/detection apparatus utilizing electrophoresis as shown in Figure 7.

(1) Pretreatment of inner walls of quartz capillary

A quartz capillary having an inner diameter of 0.1 mm and an outer diameter of 0.22 mm (O.D. 220 μm, I.D. 100 μm, SGE: Australia) was cut into capillaries having a length of 30 cm, immersed into a solution of functional regents (3-methacryloxypropyltrimethoxysilane etc.) and left for several hours. Then, the inside of the capillaries was washed with water and methanol.

(2) Charging of acrylamide

An acrylamide solution [TBE buffer (100 mM Tris borate, pH 8.0, 0.2 mM EDTA), 7M urea, 6% acrylamide, 0 to 5% bisacrylamide] was prepared, degassed by a vacuum machine or the like, added with ammonium persulfate (final concentration: 0.05%) and tetramethylethylenediamine (final concentration: 0.01%), and charged into the above quartz capillaries using an injec-

tion syringe. The capillaries were left for several hours at 4°C for completion of gelation.

(3) Preparation of template DNA

M18 primer (0.5 pmol), template DNA (M13 phage, 0.5 pmol), Sequenase Ver. 2.0 buffer (2 μl, 200 μM Tris-Cl, pH 7.5, 100 mM $MgCl_2$, 250 mM NaCl) were introduced into a sampling tube and adjusted to 10 μl with sterile distilled water to obtain a mixture. The mixture was warmed at 65°C for 10 minutes and returned to room temperature to cause annealing of the template and the primer. Then, the solution of the annealed template/DNA-primer (10 μl) was added with 0.1 M dithiothreitol (1 μl), dNTPs mix (2 μl, 1 μM dATP, dGTP, dTTP), [α-^{32}P]dCTP (0.5 μl, 300 Ci/mmol, 10 mCi/ml, Amersham) and Sequenase Ver. 2.0 (2U, Amersham). Then the reaction mixture (2.5 μl) was added to a termination mixture (3.5 μl, 80 μM dATP, dGTP, dCTP, dTTP, 8 μM ddTTP, 50 mM NaCl), mixed and allowed to react at 37°C for 10 minutes. The reaction mixture was mixed with 10 μl of formamide dye [95% formamide, 10 mM EDTA, 0.05% Bromophenol Blue (BPB), 0.05% xylene cyanol (XC)] and heated to 80°C for 5 minutes.

(4) Injection of samples and electrophoresis

A polyether sulfone ultrafiltration membrane was fused by an adhesion method such as ultrasonic bonding to a bottom surface (to be contacted with buffer) of a plate having 384 (16 x 24) through-holes having an inner diameter of 3.5 mm. The sample solution prepared as above was injected to each through-hole of the plate so that the sample solution should contact with the ultrafiltration membrane.

The plate 1 with the membrane containing the sample solution was connected with an aspirator as shown in Fig. 8 and aspirated by vacuuming from the membrane side for 10 minutes to reduce the pressure so that unreacted low molecular compounds were removed.

Then, each one terminals 6b of the above-prepared capillaries 6 charged with acrylamide gel was immersed into a TBE buffer (not shown) and the other terminal was immersed into the sample solution 7 as shown in Figure 9. The ultrafiltration membrane 5 provided on the bottom surface of the plate 1 was immersed into a TBE buffer. A voltage of 3 kV was applied between the cathode electrophoretic bath 34 and the anode electrophoretic bath 35 for 30 seconds as shown in Figure 7.

Then, the terminals of the capillaries immersed in the samples were transferred to a TBE buffer and electrophoresis was performed at 3000 V for 1 hour.

(5) Autoradiography

When BPB reached the bottom ends of the capillaries, electrophoresis was finished, and the migration pattern was analyzed by BAS 2000 imaging analyzer (FUJI). The result is shown in Fig. 10. A sequencing ladder was obtained by the above protocol as shown in Fig. 10.

According to the present invention, a means capable of easily charging reaction solutions into a large number of wells in a short time can be provided.

According to the present invention, a method for easily removing unreacted labeling substances and the like in a short time from a plurality of DNA sequencing samples containing the labeling substances and the like can also be provided.

According to the present invention, a means capable of easily transferring a large number of DNA sequencing samples to electrophoresis capillaries in a short time can be also provided.

In addition, according to the present invention, a method for easily removing unreacted labeling substances and the like in a short time from a plurality of DNA sequencing samples containing the labeling substances and the like and capable of easily transferring a large number of DNA sequencing samples to electrophoresis capillaries in a short time can also be provided.

Claims

1. A part for assembling reaction vessels comprising a base plate with through-holes passing said plate in the direction of thickness.

2. The part of claim 1 wherein the inner-diameter of the through-holes ranges from 0.01 to 10 mm.

3. A method for preparation of reaction vessels containing a reaction solution characterized in that the part of claim 1 or 2 is immersed in a reaction solution to charge the through-holes with the reaction solution and then one of apertures of each hole of the above through-holes is sealed with a membrane to complete said reaction vessels.

4. The method of claim 3 wherein the reaction solution is a buffer solution containing an enzyme.

5. A plate used for transferring DNA sequencing samples to a separation/detection system characterized in that the plate comprises a base plate with through-holes passing said plate in the direction of thickness and a membrane for sealing one of apertures of each hole of said through-holes.

6. The plate of claim 5 wherein the membrane is an ultrafiltration membrane.

7. A method for removal of unreacted low molecular compounds contained in DNA sequencing samples characterized in that said DNA sequencing samples are charged in the through-holes of the plate of claim 5 respectively, a pressure difference is provided between the remaining apertures of the through-holes and the outside of the sealing mem-

brane in such a manner that the membrane side has negative pressure to transfer said unreacted low molecular compounds in the DNA sequencing samples to the outside of the samples through said membrane.

8. A method for removal of unreacted low molecular compounds contained in DNA sequencing samples characterized in that said DNA sequencing samples are charged in the through-holes of the plate of claim 5 respectively, and a potential is applied between said DNA sequencing samples and the outside of the sealing membrane to transfer said unreacted low molecular compounds in the DNA sequencing samples to the outside of the samples through said membrane.

9. The method of claim 8 wherein terminals of electrophoretic capillaries are inserted into the DNA sequencing samples respectively, wherein the other terminals of said capillaries are contacted with an electrolyte having an electrode (1); the sealing membrane are contacted with an electrolyte with which an electrode (2) is contacted; and a potential is applied between the electrodes (1) and (2); or electrodes are inserted into the DNA sequencing samples respectively; the sealing membrane are contacted with an electrolyte with which an electrode is contacted; and a potential is applied between the electrodes inserted in the DNA sequencing samples and the electrode contacted with the electrolyte.

10. The method of any of claims 7-9 wherein the unreacted low molecular compounds are unreacted fluorescence labeling compounds.

11. The method of any of claims 7-10 wherein said DNA sequencing samples are products of DNA sequencing reactions conducted in the through-holes of the plate of claim 5, or are products of DNA sequencing reactions conducted on a micro-titer plate.

12. A method for transferring DNA sequencing samples respectively charged in the through-holes of the plate of claim 5 to electrophoretic capillaries for a separation/detection system wherein one of terminals of each capillary of the electrophoretic capillaries are inserted into said DNA sequencing samples and a potential is applied between said electrophoretic capillaries and the outside of the sealing membrane to transfer said DNA sequencing samples to the electrophoretic capillaries.

13. The method of claim 12 wherein said DNA sequencing samples are products of DNA sequencing reactions conducted in the through-holes of the plate of claim 5, or are products of DNA sequencing reactions conducted on a micro-titer plate.

14. A method for transferring DNA sequencing samples to a separation/detection system characterized in that said DNA sequencing samples are charged in the through-holes of the plate of claim 5 respectively, one of terminals of each capillary of the electrophoretic capillaries are inserted into said DNA sequencing samples contained in the through-holes from unsealed apertures and the charged contents are aspirated to the electrophoretic capillaries from the other terminal of each capillary of the electrophoretic capillaries to transfer target substances contained in said DNA sequencing samples to the electrophoretic capillaries.

15. A method for purification and transfer to a separation/detection system of DNA sequencing samples, which comprises removing unreacted low molecular compounds contained in the DNA sequencing samples and then transferring target substances contained in the DNA sequencing samples to electrophoretic capillaries for the separation/detection system, characterized in that said DNA sequencing samples are charged in the through-holes of the plate of claim 5 respectively, one of terminals of each capillary of electrophoretic capillaries are inserted into said DNA sequencing samples, a potential is applied between the outside of said membrane sealing the through-holes charged with said DNA sequencing samples and the other terminal of each capillary of the electrophoretic capillaries to transfer unreacted low molecular compounds contained in said DNA sequencing samples to the outside of the samples through the membrane and then a potential is applied between the outside of said membrane, and the other terminal of each capillary of the electrophoretic capillaries to transfer said target substances in the DNA sequencing samples into the electrophoretic capillaries.

16. A method for purification and transfer to a separation/detection system of DNA sequencing samples, which comprises removing unreacted low molecular compounds contained in the DNA sequencing samples and then transferring target substances contained in the DNA sequencing samples to electrophoretic capillaries for the separation/detection system, characterized in that said DNA sequencing samples are charged in the through-holes of the plate of claim 5 respectively, a pressure difference is provided between the remaining apertures of the through-holes and the outside of the sealing membrane in such a manner that the membrane side should have negative pressure to transfer said unreacted low molecular compounds in the DNA sequencing samples to the outside of the samples through said membrane, then one of terminals of each capillary of electrophoretic capillaries are

inserted into said DNA sequencing samples, and a potential is applied between the outside of said membrane sealing the through-holes charged with said DNA sequencing samples and the other terminal of each capillary of the electrophoretic capillaries to transfer target substances contained in said DNA sequencing samples to the electrophoretic capillaries.

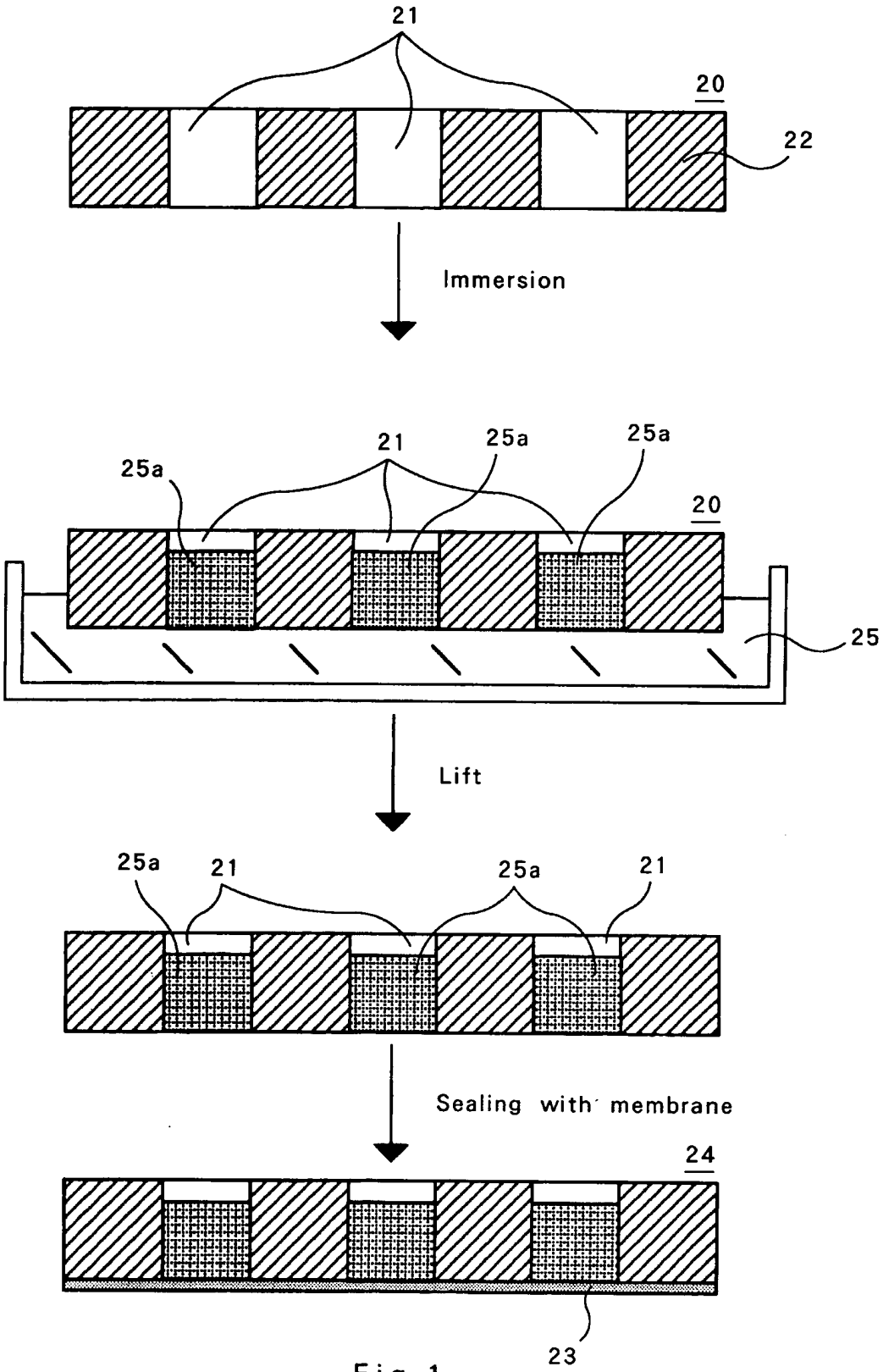
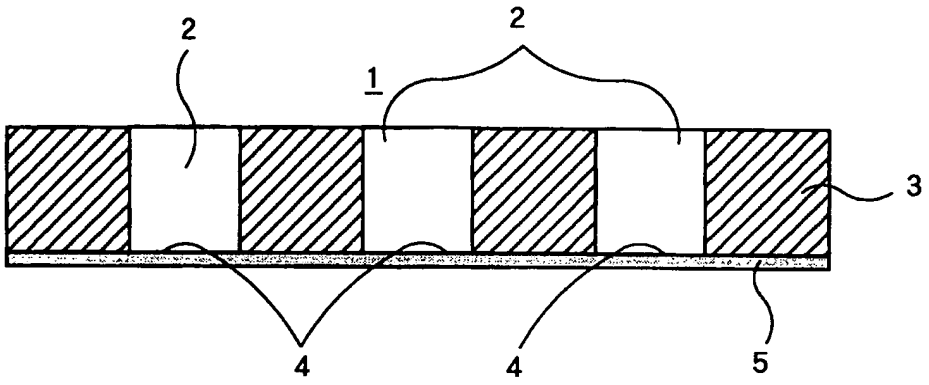

21
20
22
Immersion
21
25a
25a
25a
20
25
Lift
25a
21
25a
21
Sealing with membrane
24
23

Fig.1


2
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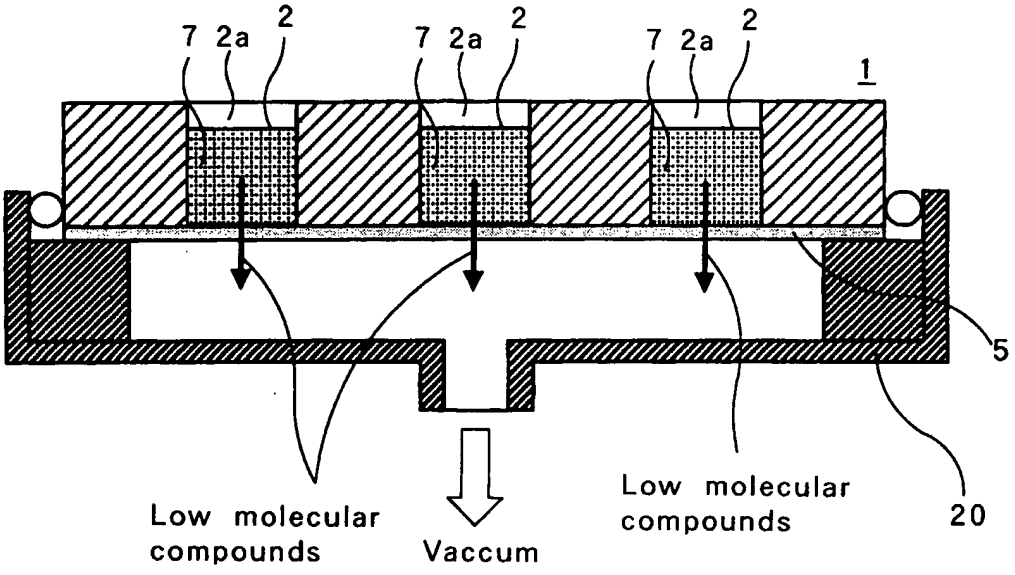
1
3
4
4
5

Fig.2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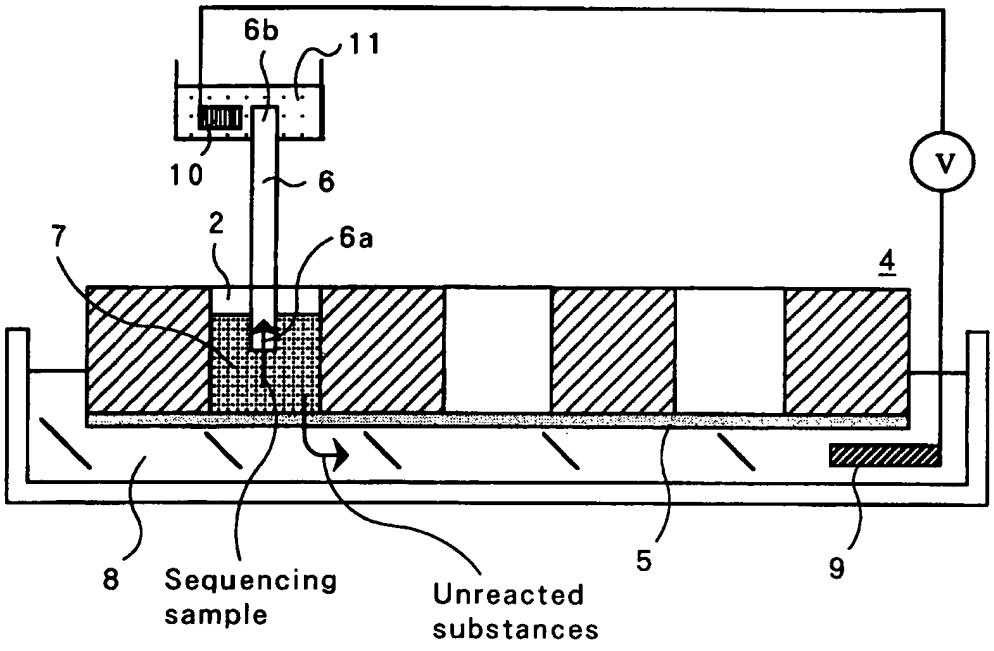
2a
2
7
2a
2
7
2a
2
1
5
Low molecular compounds
Vaccum
Low molecular compounds
20

Fig.3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11
10
6
V
7
2
6a
4
8
Sequencing sample
Unreacted substances
5
9

Fig.4

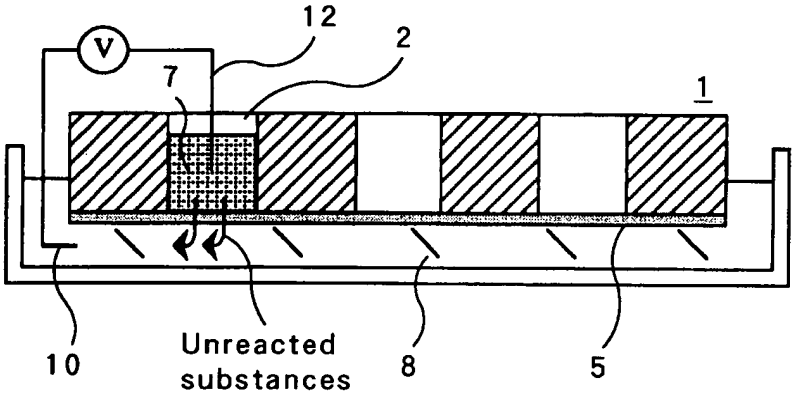
V
12
2
7
1
10
Unreacted substances
8
5

Fig.5

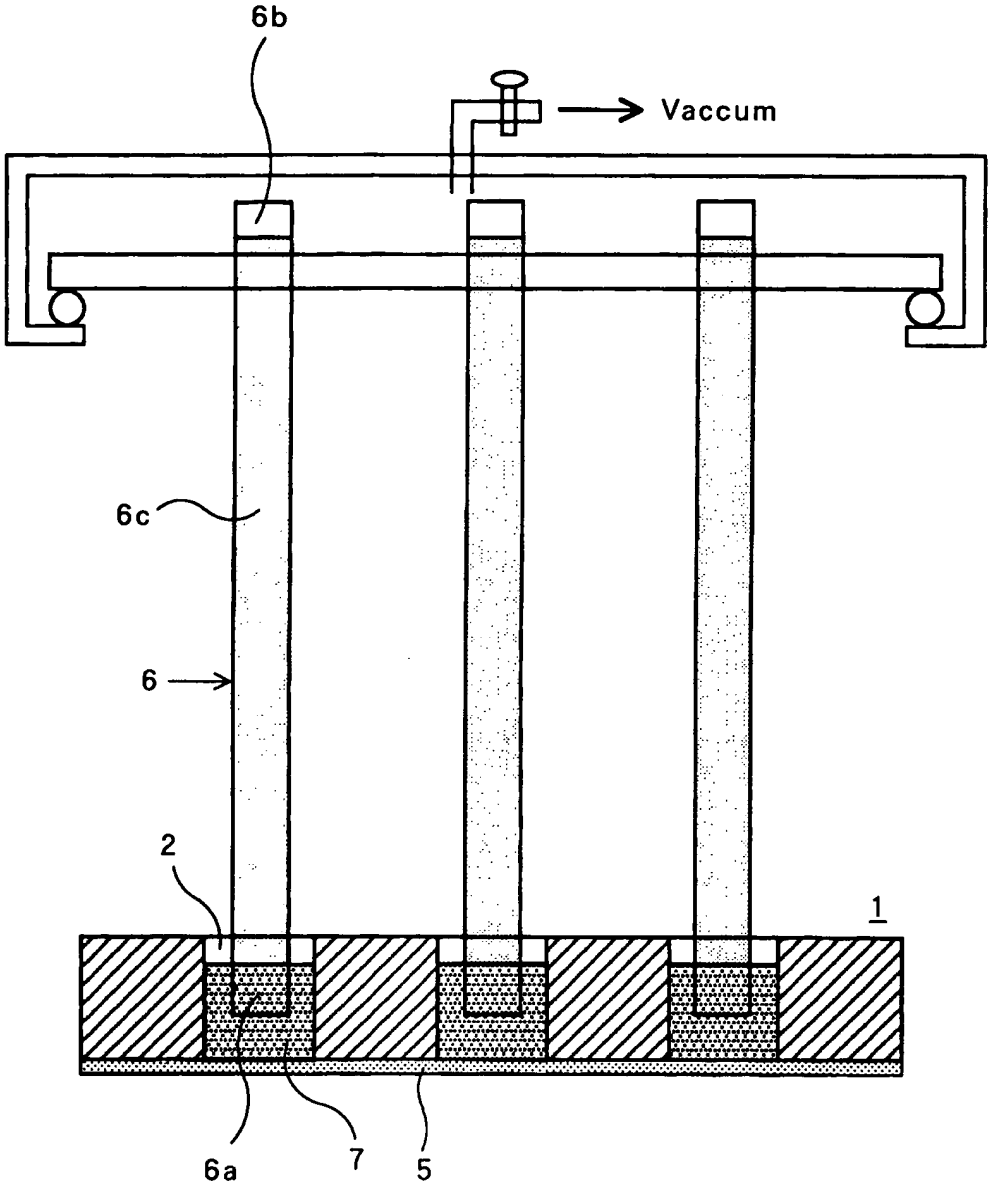

Fig.6

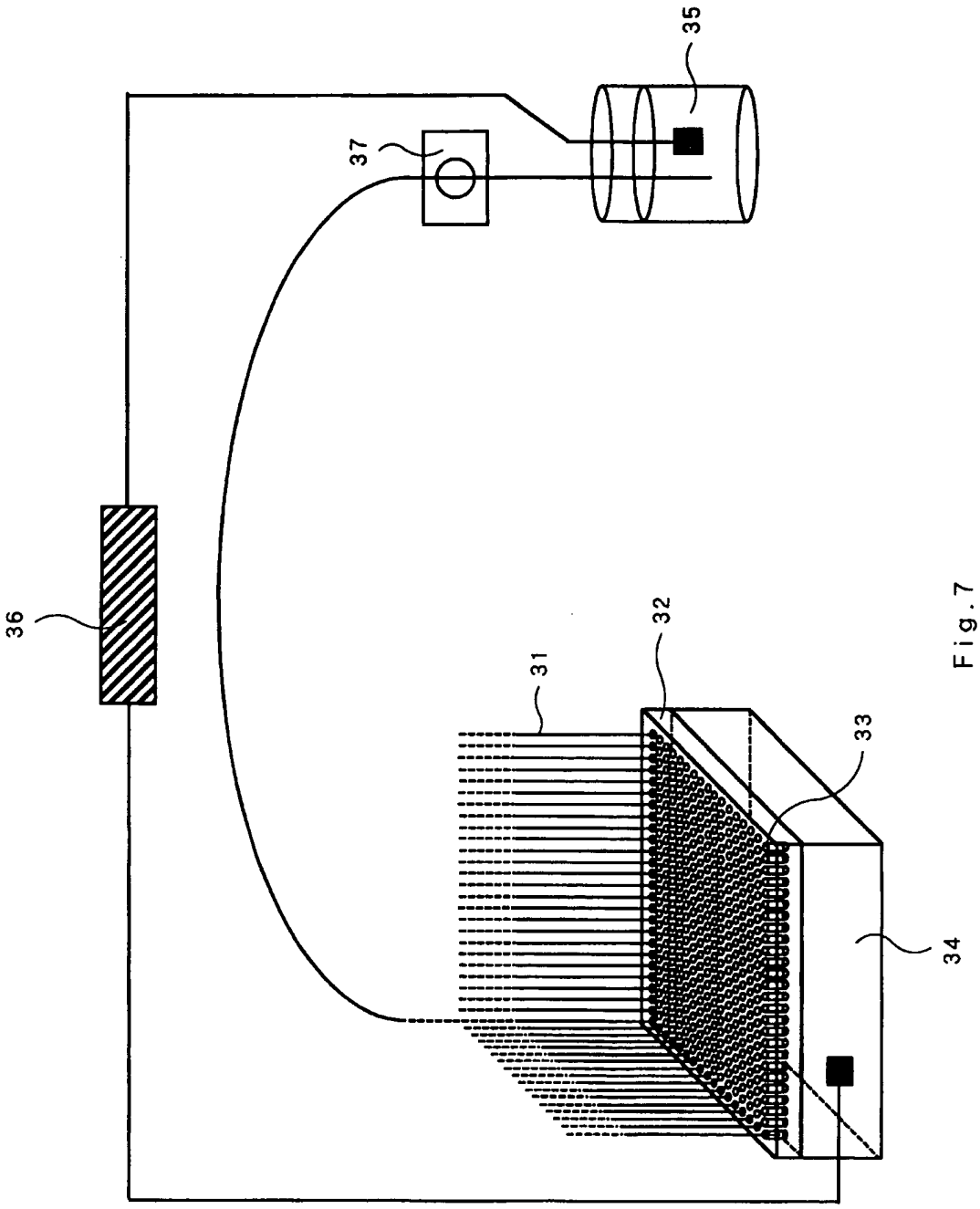

Fig.7

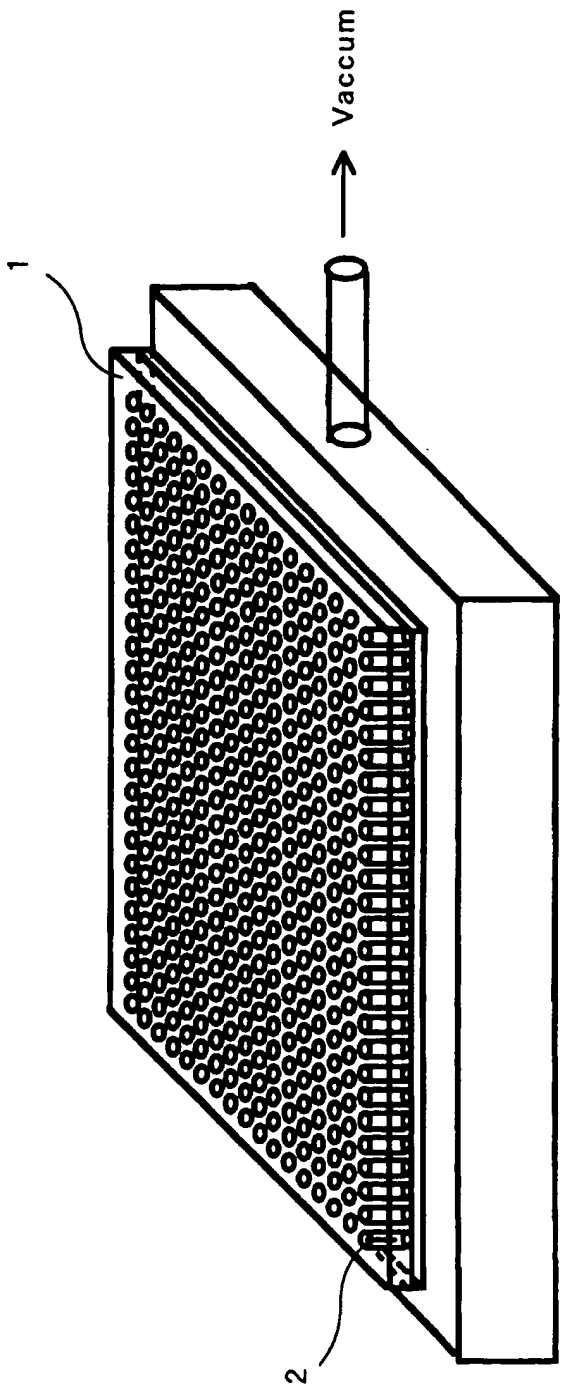

Fig.8

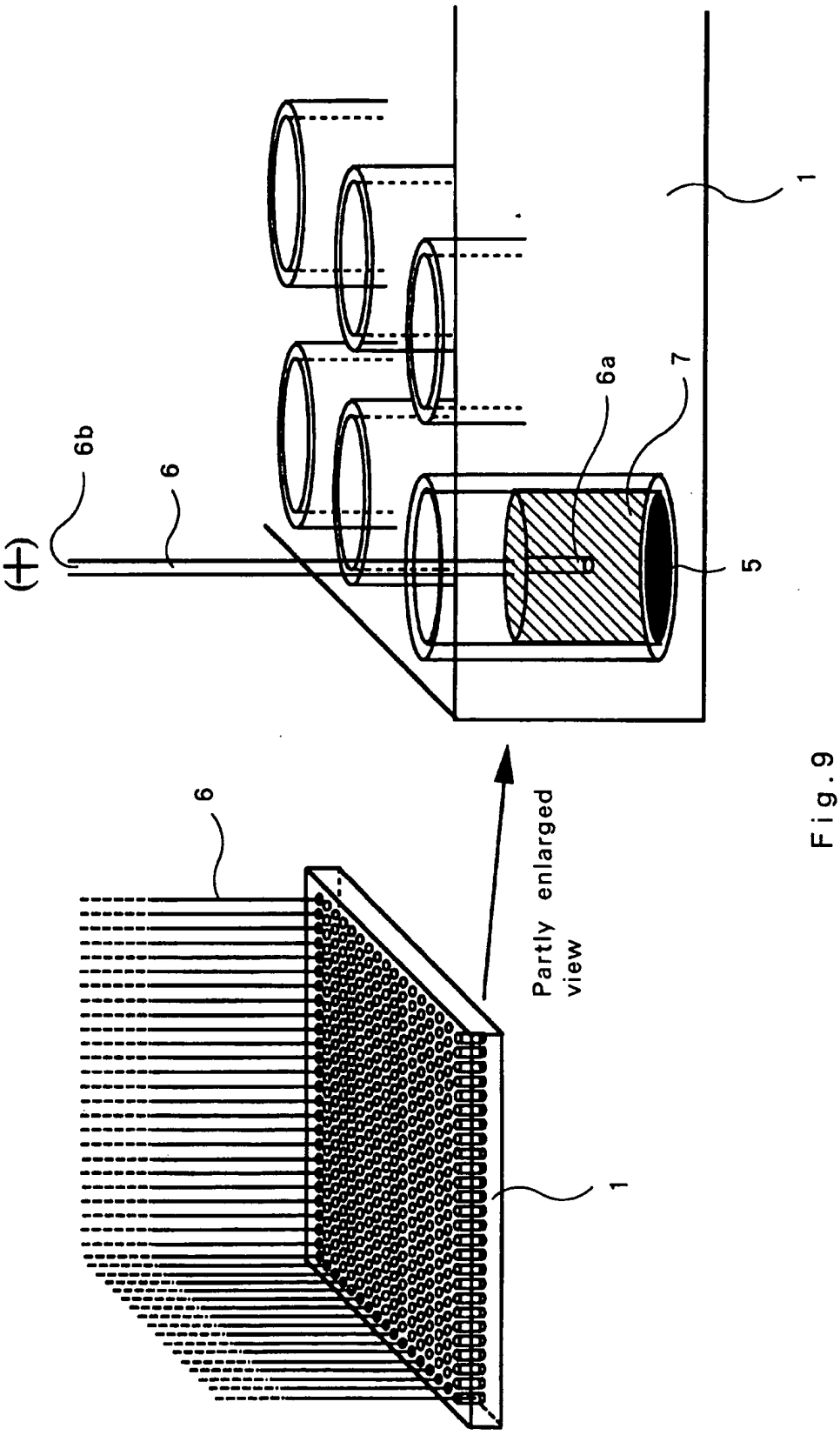

Fig.9

Fig.10

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 96 11 9652

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Y	WO 88 06723 A (BIONIQUE LABORATORIES, INC.) * abstract; figure 2 *	1	B01L3/00 G01N27/447
Y	EP 0 131 934 A (BAXTER HEALTHCARE, CORP.) * page 15, line 21 - line 30; claim 1; figure 1 *	1	
A	WO 94 29712 A (UNIVERSITY OF ALBERTA) * abstract; figure 2 *	1	
A	US 5 415 758 A (A. M. COMEAU) * abstract; figure 5 *	1	
A	ANALYTICAL CHEMISTRY, vol. 64, no. 18, 15 September 1992, pages 2149-2154, XP000319973 XIAOHUA C. HUANG: "DNA SEQUENCING USING CAPILLARY ARRAY ELECTROPHORESIS" * abstract; figure 1 *	1	
A	EP 0 581 413 A (BECKMAN INSTRUMENTS, INC.) * abstract; figure 1 *	1	TECHNICAL FIELDS SEARCHED (Int.Cl.6) B01L G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	12 March 1997	Duchatellier, M

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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L : document cited for other reasons

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